CAS No.: 71-43-2
Robust Summary No.: OP E-002

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Biodegradation

Test Substance:	CAS No. 71-43-2; Benzene
Method/Guideline:	OECD 301F
Year (guideline):	1993
Type (test type):	Ready Biodegradability, Manometric Respirometry Test
GLP:	Yes
Year (study performed):	2000
Inoculum:	Domestic activated sludge
Exposure Period:	28 days

Test Conditions: (FT - TC)

- **Note: Concentration prep., vessel type, replication, test conditions.**

Activated sludge and test medium were combined prior to test material addition. Test medium consisted of glass distilled water and mineral salts (Phosphate buffer, Ferric chloride, Magnesium sulfate, Calcium chloride, EDTA).
Test vessels were 500 mL dark glass bottles placed on a magnetic stirrer and electronically monitored for oxygen consumption.
Test material and blanks were tested in triplicate, controls were tested in duplicate.
Test material (benzene) concentration was 17mg/L. Sodium benzoate (positive control) concentration was 30mg/L. Toxicity control with benzene and Na Benzoate concentrations at 17 and 30 mg/L, respectively.
Test temperature was 22 +/- 2 Deg C.
All test vessels were stirred constantly for 28 days using magnetic stir bars and plates.

Results: (FT - RS)

Units/Value:

- **Note: Deviations from protocol or guideline, analytical method.**

Test material was readily biodegradable. Half-life was <2 weeks.
By day 28, 63.0% degradation of the test material was observed.
10% biodegradation was achieved in less than 5 days, 50% biodegradation on approximately day 5.
By day 5, >60% biodegradation of positive control was observed, which meets the guideline requirement. No excursions from the protocol were noted.
Biodegradation was based on oxygen consumption and the theoretical oxygen demand of the test material as calculated using results of an elemental analysis of the test material.

Sample	% Degradation* (day 28)	Mean % Degradation (day 28)
Benzene	54, 72, 63	63
Na Benzoate	65, 75	70
Toxicity Control	59, 65	62

* replicate data

Conclusion: (FT - CL)

Biodegradation

Reliability: (FT - RL)	(1) Reliable without restriction
Reference: (FT - RE)	Brixham Environmental Laboratory. 2001. OECD 301F, Ready biodegradability: Manometric respirometry. Study # AH0378/A.
Other (source): (FT - SO)	Olefins Panel, American Chemistry Council

* IUCLID field abbreviations include:
FT - Freetext
TC - Test Conditions
RS - Results
CL - Conclusion
RL - Reliability
RE - Reference
SO - Source

Robust Summary - Group 5: High Benzene Naphthas

Acute Toxicity

Test Substance	Dripolene. Yellow, homogeneous liquid, stable for 5 years at ambient temperature. (CRU #93329). Olefins Panel HVP Stream: Pyrolysis Gasoline. Typical composition ranges for Pyrolysis Gasoline are shown in Table 2 of the Test Plan.
Method	
Method/guideline followed	Not specified
Type (test type)	Acute, limit test
GLP	Yes
Year	1994
Species/Strain	Rat, Sprague-Dawley
Sex	Males and females
No. of animals per sex per dose	5
Vehicle	None
Route of administration	Oral gavage
Test Conditions	Sprague Dawley rats (180-350g) were individually housed in stainless steel suspended cages and fasted overnight prior to administration of 2g/kg neat dripolene. The study room was maintained at 68-72^{0}F with a relative humidity of 35-63% and a 12 hr light-dark cycle. Water and chow diet were available ad lib after dosing. Test article was administered once on day 1 by oral gavage through a blunted needle. Rats were observed for clinical signs approx. 30 min, 1hr, and 4hr, after dosing, and daily thereafter until sacrifice on day 15. Rats were checked once a day for mortality and moribundity. Observations were not made on weekends. Body wts were recorded prior to fasting and on days 1, 8 and 15.
Results	
LD_{50} with confidence limits.	The LD_{50} was not reached at 2g/kg. There were no deaths and all rats gained some weight during the study. Clinical signs noted in one or more rats were salivation, decreased activity, rales, lacrimation, chromodacryorrhea, ataxia, head shaking, chromorhinorrhea, miosis, slight tremors, mydriasis, hyperactivity, hypothermia, urogenital discharge, nasal discharge, decreased food consumption, decreased fecal output, vocalization, and decreased stool size. No gross pathological findings were noted at necropsy.
Remarks	
Conclusions (study author)	The LD_{50} was not reached at 2g/kg.
Data Quality Reliability	**1.** Reliable without restriction.
References	Rodriguez, S.C. and Dalbey, W.E. 1994. Acute oral toxicity of dripolene in Sprague Dawley Rats. Study #65642. Stonybrook Laboratories, Princeton, NJ. for Mobil Chemical Co., Edison, NJ.
Other *Last changed*	10/23/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 5: High Benzene Naphthas

Acute Toxicity

Test Substance	Dripolene. Yellow, homogeneous liquid, stable for 5 years at ambient temperature. (CRU #93329). Olefins Panel HVP Stream: Pyrolysis Gasoline. Typical composition ranges for Pyrolysis Gasoline are shown in Table 2 of the Test Plan.
Method	
Method/guideline followed	Not specified
Type (test type)	Acute, limit test
GLP	Yes
Year	1994
Species/Strain	Rabbit, New Zealand White
Sex	Males and females
No. of animals per sex per dose	3
Vehicle	None
Route of administration	dermal
Test Conditions	Rabbits, weighing at least 2kg, were individually housed in stainless steel suspended cages in a study room maintained at 69-72^{0}F with a relative humidity of 40-85% and a 12 hr light-dark cycle. Water and chow diet were available ad lib. The dorsal skin surface extending down from the front to rear legs and from left to right lower flanks was clipped free of hair the day prior to test article administration. Test article was spread evenly over the clipped area (approx. 10% of body surface area) at a dose of 2g/kg. A layer of 8-ply gauze was placed on the dorsal site, and a rubber dam sleeve was fitted snugly over the gauze pad and around the trunk. Edges of the dam were taped in place. An Elizabethan collar was affixed to the neck to prevent oral ingestion of test article and mechanical irritation of the test site. After 24 hrs, the collar and wrappings were removed and residual test article was wiped off. Body wts were recorded on days 1, 8 and 15. Rabbits were observed for toxicity at about 1 and 2 hr post-dose and daily thereafter on weekdays, through day 14. Observations for mortality/moribundity were made daily. Rabbits were sacrificed on day 15 and necropsies were performed.
Results	
LD_{50} with confidence limits.	The LD_{50} was not reached at 2g/kg. There were no deaths during the study and rabbits either gained some weight or remained at day 1 body wt. Signs that might have resulted from treatment in one or more rabbits were: decreased fecal output, decreased fecal pellet size, soft stool, and decreased food consumption. No gross pathological findings were noted at necropsy.
Remarks	
Conclusions	
(study author)	The LD_{50} was not reached at 2g/kg.
Data Quality	
Reliability	**1.** Reliable without restriction.
References	Rodriguez, S.C. and Dalbey, W.E. 1994. Dermal toxicity of dripolene in the New Zealand White rabbit. Study #65643. Stonybrook Laboratories, Princeton, NJ. for Mobil Chemical Co., Edison, NJ.
Other	
Last changed	10/23/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 5: High Benzene Naphthas

Acute Toxicity

Test Substance	Hydrogenated Pyrolysis Gasoline CAS# 68410-97-9. Clear liquid, aromatic odor. Olefins Panel HVP Stream: Hydrotreated C6-C8.
Method	
Method/guideline followed	Standard method (not referenced) with doses based on a limit test and range-finding study
Type (test type)	Acute LD50
GLP	Yes
Year	1984
Species/Strain	Rat, Fischer 344
Sex	Males and females
No. of animals per sex per dose	5
Vehicle	None
Route of administration	Oral
Test Conditions	Rats (99.9-134.0 g; 57 days old) were individually housed in screen-bottomed cages in a room with 70.6^{0}F temperature, relative humidity of 59% and a 12 hr light/dark cycle. Chow diet and tap water from an automatic watering system were available ad lib. Rats were fasted for 24 hours prior to dosing at 4.2, 4.6, 5.0, and 5.4g/kg and observed at 1 and 4 hrs after dosing on day 1, and daily thereafter, over 14 days for clinical signs, morbidity and mortality. Gross necropsies were performed on all rats. LD50 was calculated by Probit analysis.
Results	
LD_{50} with confidence limits.	LD50 = 5.17g/kg (95% confidence limits: 5.02-5.45g/kg)
Remarks	On day 1, males and females showed dose responsive increases in ataxia, harsh respiratory sounds, and a non-dose responsive increase in red ocular discharge. Soft feces were observed in treated males and females on day 2. Frequency of clinical signs decreased by day 3 and signs were absent by day 5. There were no changes in body weight gain among the groups. Male and female mortalities were combined to calculate an LD50. Mortality from a previously performed limit test, conducted at 5.0g/kg was combined with results from the 5.0g/kg dose in this definitive study, raising that group number to 20. Mortalities were: 0/10 at 4.2, and 4.6g/kg, 7/20 at 5.0g/kg, 7/10 at 5.4g/kg. Gross necropsies revealed red lungs, gas-filled stomach and intestine, mottled liver, discoloration of kidney, and opaque eyes in rats that died during the study. These observations, with the exception of opacity in the left eye of one 5.4g/kg female, were absent in rats sacrificed at study termination (day 15).
Conclusions (study author)	The acute median lethal dose (LD50) for Hydrogenated Pyrolysis Gasoline in male and female rats was 5.17g/kg. A descriptive classification of Practically Non-toxic for acute oral exposure was assigned.
Data Quality	
Reliability	**1.** Reliable without restrictions.
References	Rausina, G.A. 1984. Acute oral toxicity study in rats of hydrogenated pyrolysis gasoline. Proj. #2091. Gulf Life Sciences Center, Pittsburgh, PA
Other	
Last change	5/7/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 5: High Benzene Naphthas

Acute Toxicity

Test Substance	Hydrogenated Pyrolysis Gasoline CAS# 68410-97-9. Clear liquid, aromatic odor. Olefins Panel HVP Stream: Hydrotreated C6-C8.
Method	
Method/guideline followed	Standard method (not referenced)
Type (test type)	Acute LC50
GLP	Yes
Year	1984
Species/Strain	Rat, Fischer 344
Sex	Males and females
No. of animals per sex /dose	5
Vehicle	Filtered air
Route of administration	Inhalation
Test Conditions	Rats (8 wks. old, 100-172g at initiation) were individually housed in stainless steel, screen-bottomed cages in a room maintained at 73.0^0F (75.5^0F during exposure) temperature, relative humidity of 51% (40% during exposure) and a 12 hr light/dark cycle. Rats received chow diet and tap water ad lib, except during exposure. One group of 10 rats was exposed to aerosolized test article generated by a ball jet nebulizer for 4 hrs. A condensing flask was used to prevent large particles from entering the chamber. Actual average chamber concentration was 12,408ppm (range 8,642-17,371ppm) determined by gas chromatography. Particulate phase was negligible. Rats were observed for clinical signs at 1 and 4 hrs after dosing on day 1 and daily thereafter over 14 days, and for morbidity and mortality twice daily on weekdays, once daily on weekends. Body wt. was determined at initiation and on days 8 and 15. Gross necropsies were performed on all rats at termination on day 15.
Results	
LC_{50} with confidence limits.	LC50>12,408ppm
Remarks	There were no deaths during the study, no effects on body wt gain, and no gross alterations were seen at necropsy. Immediately after exposure, all rats exhibited lethargy, increased and labored respiration, and ocular discharge; most animals showed twitching and dry red material around nose/mouth. There were a few instances of harsh respiratory sounds, trembling, and perianal soiling. These clinical signs decreased in frequency by 4 hr post-exposure and disappeared by day 2.
Conclusions (study author)	No deaths occurred at the dose of 12,408ppm of test article, indicating a descriptive classification of Practically Non-toxic for acute inhalation exposure. Clinical signs noted immediately after exposure (increased/labored respiration, twitching, trembling, lethargy, ocular discharge) were not observed by day 2 and thereafter.
Data Quality Reliability	**1.** Reliable without restrictions.
References	Rausina, G.A. 1984. Acute inhalation toxicity study in rats of hydrogenated pyrolysis gasoline. Proj. #2092. Gulf Life Sciences Center, Pittsburgh, PA
Other	
Last change	Revised 7/27/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 5: High Benzene Naphthas

Genetic Toxicity - in Vitro

Test Substance	
Test substance	Hydrogenated Pyrolysis Gasoline, CAS #68410-97-9. clear liquid with aromatic odor, negligible solubility in water, contains <55.0% benzene, <25% toluene, <10% dimethyl benzene/xylene, <7% pentane, <7% ethylbenzene, <3% cyclohexane, <2% hexane. Olefins Panel HVP Stream: Hydrotreated C6-C8.
Method	
Method/guideline followed	Standard method per Ames et al
Type	Reverse mutation bacterial assay
System of testing	Salmonella typhimurium, Escherichia coli with and without metabolic activation
GLP	Yes
Year	1991
Species/Strain	S. typh. TA1535, TA1537, TA98, TA100; E. coli WP2(uvrA)
Metabolic activation	Yes
Species and cell type	Male Sprague Dawley rat liver (S9 fraction), Molecular Toxicology, Inc., Annapolis, MD
Quantity	20% S9 fraction in 0.5ml S9 mix/plate
Induced or not induced	Aroclor 1254induced, rats given a single 500mg/kg ip dose
Concentrations tested	0, 33, 100, 333, 1000, 3333, 10,000µg/plate ± S9. All diluted in acetone (200mg/ml)
Statistical Methods	None specified. Test article considered mutagenic when it induces a reproductive, dose-related increase in number of revertants in one or more strains at 3 consecutive dose levels. A non-mutagen does not induce a dose-related increase in at least 2 independent tests.
Remarks for Test Conditions	Hydrogenated pyrolysis gasoline (HPG) was prepared in acetone immediately prior to use. At end of the study, an aliquot of the stock dilution was sent to PTRL West, Richmond, CA to confirm concentration. Salmonella (approx. 10^8cells/ml) were exposed to either test material or acetone in 3 plates/dose ± S9 by the plate incorporation method. Six dose levels from 33-10,000µg/plate were employed in both the range-finding trial using TA100 and the mutagenicity test with all strains of Salmonella and E. coli. Optimum level of S9 for the mutagenicity assay was determined by testing the highest non-toxic dose, 10,000µg per plate with metabolic activation systems containing 4, 20 or 80% S9 fraction. No noteworthy increases in revertants or cytotoxicity was observed at any S9 concentration; 20% S9 was used in the mutagenicity test. All plates were incubated at 37^0C for 48 hrs then revertant colonies were counted. Positive control compounds were: cultures-S9, sodium azide (5µg/plate) for TA1535, TA100; 9-aminoacridine (50µg/plate) for TA1537; 2-nitrofluorene (5µg/plate) for TA98; N-ethyl-N'-Nitro-N-Nitrosoguanidene (5ug/plate) for E. coli WP2, and cultures+S9, 2-anthramine (4µg/plate) for TA1535, TA1537, (2µg/plate) for TA98, TA100, and (20µg/plate) for E. coli WP2. Two independent assays were performed.
Results	
Genotoxic effects	HPG did not induce increases in number of revertant colonies and no toxicity was observed in any Salmonella strain or E. coli WP2 with or without 20% S9 metabolic activation in both studies. Positive control compounds performed appropriately.
Conclusions	
(contractor)	Hydrogenated pyrolysis gasoline is not mutagenic to bacteria under conditions of this assay.
Data Quality	
Reliabilities	**1**. Reliable without restriction
Reference	Riccio, E.S. and Stewart, K.R. 1991. Salmonella-Escherichia coli/microsome plate incorporation assay of Hydrogenated Pyrolysis Gasoline. SRI Study #2545-A03-91, Sponsor study #91-66. SRI International, Menlo Park, CA for Chevron Environmental Health Center, Richmond, CA
Other	
Last changed	5/7/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 5: High Benzene Naphthas

Genetic Toxicity - in Vitro

Test Substance	Hydrogenated Pyrolysis Gasoline, CAS #68410-97-9. clear liquid with aromatic odor.
Test substance	Composition, purity and stability referred to sponsor. Olefins Panel HVP Stream: Hydrotreated C6-C8.
Method	
Method/guideline followed	Standard method based on Cortesi et al (1983), Dunkel et al (1981), Reznikoff et al (1973)
Type	In vitro cell transformation
System of testing	Mouse embryo cells
GLP	Yes
Year	1984
Species/Strain	BALB/3T3-A31-1-1 from T. Kakunaga, National Cancer Inst., 1983
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	Cytotoxicity: 8, 16, 32, 64, 128, 256, 512, 1024, 2048, and 5000µg/ml; Transformation: 100, 250, 500, 1500µg/ml, all diluted in 10% Pluronic® polyol F68 (prepared in deionized water, mol. wt. 8350, 80% hydrophilic).
Exposure period	2 days
Statistical Methods	None employed. Criteria for positive response were a two-fold increase in type III foci at the highest dose over vehicle control (at least 2 type III foci if vehicle control had none) with or without a dose related response, or a two-fold increase at two or more consecutive doses. Test is equivocal if two-fold increase occurred at any one level other than the highest acceptable dose.
Remarks for Test Conditions	Sufficient Hydrogenated Pyrolysis Gasoline (HPG) was weighed separately for each dose level, 0.40ml of 10% F68 added per ml of final volume and medium (Eagles MEM with 10% heat-inactivated fetal calf serum) added as required to achieve final volume for testing. Test preparations were mixed just prior to addition to cultures at 50µl to each 5 ml culture. All cultures were incubated at 37^0C in 5% CO_2 enriched humidified atmosphere. For cytotoxicity, 2 cultures/dose group, 2 cultures for vehicle F68 or medium negative control were seeded with $1x10^4$ cells/plate in day 1, exposed on days 2-3, trypsinized and counted with a Coulter Model ZB on day 4 for at least 20% survival. For transformation, 15 cultures ($1x10^4$ cells/flask/dose group)) and two colony-forming cultures (100 cells/plate/dose group) were seeded on day 1, exposed on days 2-3 and culture medium changed on day 4. For transformation cultures, medium continued to be changed weekly to day 29. Positive control was 3-methylcholanthrene (1µg/ml). Colony forming cultures were fixed, stained, and counted visually on day 10 to determine cloning efficiency (avg. number colonies/plate ÷ 100 cells seeded). Transformation cultures were fixed and stained on day 29 for focus counting and evaluation. Transformation frequency = total type III foci ÷ total flasks/dose group.
Results	
Genotoxic effects	HPG induced toxicity in BALB/3T3 cells after two days exposure beginning at 128 µg/ml (45.4% relative survival) with relative survivals of 26.7, 25.6, 3.2 and 0% at 512, 1024, 2048 and 5000µg/ml, respectively. In the transformation assay, toxicity was seen at all dose levels (relative cloning efficiencies of 53.7, 67.8, 78.5 and 0% at 100, 250, 500 and 1500µg/ml). At 1500µg/ml, the highest dose level, HPG induced 5 Type III foci; no other dose levels produced a positive response. Transformation frequencies were 0.13, 0, 0, 0.07 and 0.36 for medium control, vehicle control, 100, 250, 500 and 1500µg/ml, respectively. Positive and negative controls gave appropriate responses.
Conclusions	
(contractor)	Hydrogenated Pyrolysis Gasoline induced transformation in BALB/3T3 cells under conditions of this assay. Cytotoxicity and impairment of cloning efficiency were also observed. The positive response was observed only at the highest dose level, a level that appeared to be too toxic for cells to recover and form colonies (0% relative colony forming efficiency)

Data Quality *Reliabilities*	**1.** Reliable without restriction
Reference	Brecher, S. 1984. Transformation test of Hydrogenated Pyrolysis Gasoline. Proj. #2098. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co, Houston, TX Cortesi, E. et al. 1983. Teratogenesis, Carcinogenesis, Mutagenesis 3: 101-110. Dunkel, V.A. et al. 1981. J. Nat'l Cancer Inst. 67: 1303-1315. Reznikoff, C.A. et al. 1973. Cancer Res. 3239-3249.
Other *Last changed*	Revised 8/27/2001 (Prepared by a contractor to the Olefins Panel).

Robust Summary - Group 5: High Benzene Naphthas

Genetic Toxicity - in Vitro

Test Substance	Hydrogenated Pyrolysis Gasoline, CAS #68410-97-9. clear liquid with aromatic odor.
Test substance	Composition, purity and stability referred to sponsor. Olefins Panel HVP Stream: Hydrotreated C6-C8.
Method	
Method/guideline followed	Standard method based on Williams et al (1977, 1982)
Type	In vitro mammalian DNA repair assay
System of testing	Unscheduled DNA synthesis (UDS) in primary hepatocyte cultures
GLP	Yes
Year	1984
Species/Strain	Fischer 344 male rat (10 wks old)
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	8, 16, 32, 64, 128, 256, 512, 1024µg/ml diluted in 10% Pluronic F68 (prepared in deionized water, mol. wt 8350, 80% hydrophilic)
Exposure period	18 hrs.
Statistical Methods	None specified. Criteria for positive response are incorporation of radioactive precursor (^{3}H-thymidine) in cells that are not normally synthesizing DNA, indicating repair of damage. A positive response is defined as a mean net nuclear grain count at any treatment level that exceeds concurrent negative control by at least 6 grains/nucleus; negative control value must not exceed 5 grains. If this criterion is not met, a positive response can be identified if there is a significant difference ($p<0.01$) in % cells in repair at any dose level and negative control value. This indicator defines whether a small fraction of cells is undergoing repair (Casciano & Gaylor, 1983). A positive response need not be dose related.
Remarks for Test Conditions	Sufficient Hydrogenated Pyrolysis Gasoline (HPG) was weighed separately for each dose level, 0.40ml of 10% F68 added per ml of final volume and sufficient medium (Williams Medium E with 10% fetal bovine serum and insulin) added to achieve final volume. Test preparations were mixed just prior to addition at 20µl to each 2ml culture. The conc. of ^{3}H-thymidine (½ life 12.4 yrs.) used in these assays was 1mCi/ml. All cultures were incubated at 37^0C in 5% CO_2 enriched humidified atmosphere. No range finding assay was performed. In the UDS assay, $2x10^5$ cells/ml were seeded into coverslip cultures, exposed to ^{3}H-thymidine and test substance for 18 hours (3 cultures/dose level, 8 dose levels), untreated controls, vehicle F68 control and positive control, 2-acetyl aminofluorene (0.01µg/ml). Cells growing on coverslips were rinsed, fixed and glued to microscope slides on day 2. On day 3, slides were dipped in autoradiographic emulsion and stored in the dark at $2\text{-}8^0$C. Autoradiographs were developed, stained and coverslipped on day 10. Numbers of grains overlying 50 randomly selected nuclei/slide were counted. The highest of 3 cytoplasmic grain counts/cell were subtracted and this number was divided by a conversion factor (unspecified) to obtain net nuclear grain count. Avg. net nuclear grain count/slide (sum of net nuclear grain count ÷ 50) and mean net nuclear grain count (avg. net nuclear grain count/slide ÷3) were calculated. In addition, % cells in repair were determined for each dose level.
Results	
Genotoxic effects	HPG induced toxicity in primary hepatocytes following 18 hr exposure that left too few cells for UDS analysis at doses of 512 and 1024µg/ml. HPG did not induce unscheduled DNA synthesis at any dose level with sufficient cells to be analyzed. Positive and negative controls gave appropriate responses.
Conclusions	
(contractor)	Hydrogenated Pyrolysis Gasoline did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes under conditions of this assay.

Data Quality *Reliabilities*	**2.** Reliable with restrictions. No table of cell counts/viability. No individual data to verify calculations and identify conversion factor. Statistical criteria are mentioned but method is not cited.
Reference	Brecher, S. 1984. Hepatocyte primary culture/DNA repair test of Hydrogenated Pyrolysis Gasoline. Proj. # 2097. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX Williams, G.M. 1977. Cancer Res. 37: 1845-1851 Williams et al. 1977. In Vitro 13: 809-817 Williams et al. 1982. Mut. Res. 97:359-370 Casciano, D.A. and Gaylor, D.W. 1983. Mut. Res. 122:81-86
Other *Last changed*	5/7/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 5: High Benzene Naphthas

Genetic Toxicity - in Vivo

Test Substance	
Remarks	Hydrogenated Pyrolysis Gasoline, CAS #68410-97-9. Clear liquid with aromatic odor. Compositional analysis , purity and stability referred to sponsor. Olefins Panel HVP Stream: Hydrotreated C6-C8.
Method	
Method/guideline followed	None specified. Comparable to standard assay.
Type	Mammalian bone marrow erythrocyte micronucleus assay
GLP	Yes
Year	1984
Species	Mice
Strain	Crl:CD-1(ICR)BR Swiss
Sex	Male and female. Range-finding 2M, 2F (10 wks old)/group; 3 groups; Micronucleus test 10M, 10F (11 wks old)/group in 4 groups, 15M, 15F in one group.
Route of administration	Oral gavage
Doses/concentration levels	0, 0.5, 1.0, 2.0g/kg (2doses), 2.0g/kg (1 dose) undiluted
Exposure period	1 dose/day for 2 days: one group- 1 dose, 1 day only
Statistical methods	Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes (PCEs) with micronuclei (MN) , and group mean ratios of PCE to normochromatic erythrocytes (NORMs) were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant ($p<0.05$) increases in micronucleated PCE at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met.
Remarks for Test Conditions.	Animals in the range-finding study (2M, 2F/group), 3 treated groups (no control group) were given 1.25, 2.5, and 5.0g/kg neat hydrogenated pyrolysis gasoline (HPG) by gavage once each day for two days. Eighty percent of the dose level that produced =50% mortality was selected for the maximum dose in the micronucleus study. In the micronucleus study, three groups of mice were given undiluted HPG by oral gavage daily for two days at doses of 0.5, 1.0, 2.0g/kg, negative control mice were given corn oil (5g/kg). One-half of each treated group and negative control (5M, 5F) was killed on day 3 and the remainder on day 4. One group (15M, 15F), given 2.0 g/kg by gavage in a single dose for 1 day only, was killed on days 2, 3, 4 (5/sex/day). Positive control mice (4M, 4F) given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Survival, body wt, and clinical signs were observed and recorded daily. Slides of femoral bone marrow smears were prepared, stained with May-Grunewald/Giemsa stain and examined microscopically. For each mouse, 1000 PCE and all associated mature erythrocytes (NORMs) were counted. Data collected included group mean body weights for each day, total PCEs, total NORMs, PCEs with MN, and NORMs with MN.
Results	
Genotoxic effects	NOAELmortality = 1.0g/kg; NOELgenetics > 2.0g/kg (Assigned by reviewer)
NOAEL (NOEL)	In the range-finding study, half of the animals given HPG at conc of 5.0g/kg died on or before day 2. Gross necropsy of dead mice was unremarkable. In the micronucleus test, 1/10 males given 2.0g/kg (2 doses) died on day 2. No other mortality or significant wt changes were observed. Lethargy was observed among high dose mice. Surviving mice treated with HPG did not show any significant increase in micronucleus formation in PCE and no significant changes in ratio of PCE/NORM compared to negative controls. Negative and positive controls gave appropriate results.
LOAEL (LOEL)	
Conclusions	
(study authors)	Oral treatment of mice with Hydrogenated Pyrolysis Gasoline for 1-2 days at doses up to 2.0g/kg/day had no effect on frequency of micronucleated polychromatic erythrocytes in bone marrow under these test conditions. HPG did not induce cytogenetic damage.

Data Quality	
Reliabilities	**1.** Reliable without restriction
References	Khan, S.H. 1984. Micronucleus test of Hydrogenated Pyrolysis Gasoline. Proj. #2096. Gulf Life Sciences Center, Pittsburgh, PA for Gulf Oil Chemicals Co., Houston, TX
Other	
Last changed	5/7/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 5: High Benzene Naphthas

Repeated Dose Toxicity

Test Substance	Hydrogenated Pyrolysis Gasoline CAS #68410-97-9, Clear liquid with aromatic odor.
Remarks	Olefins Panel HVP Stream: Hydrotreated C6-C8.
Method	
Method/guideline followed	Standard method, method not referenced
Test type	Subacute
GLP	Yes
Year	1984
Species	Rat
Strain	Fischer 344
Route of administration	Inhalation
Duration of test	8 days
Doses/concentration levels	0, 4869±470, 9137±917ppm±SD, actual exposure conc.
Sex	Males and females (5/sex/group)
Exposure period	6 hrs.
Frequency of treatment	once daily for 5 days (d1-5)
Control group and treatment	5M, 5F; filtered air
Post exposure observation period	2 days
Statistical methods	Body wt variance compared by Bartlett's test and one way analysis of variance. Group mean body wt compared either with Dunnett's test or a modified t-test to assess significance.
Test Conditions	Rats (9 wks old, 113-195g at initiation) were housed individually in stainless steel, screen-bottomed cages. Rooms were maintained at 72.2^0F (exposure chamber 75^0F) with relative humidity of 54% (exposure chamber 50%), and 12 hr light/dark cycle. Rats received chow diet and tap water ad lib throughout the study, except during exposure. Three groups of 10 rats (5M, 5F/group) each, were exposed to test article or air. Test article was aerosolized with a ball jet nebulizer; an in-line condensing flask was used to prevent large particles from entering the exposure chamber. Chamber concentration of test article was measured by gas chromatography. Rats were observed twice daily on weekdays and once daily on weekends for morbidity/mortality, and once daily for clinical signs immediately after exposure on days 1-5. Surviving rats were sacrificed on day 8. Gross necropsies were performed on all rats.
Results	
NOAEL (NOEL)	NOAEL< 4869ppm (estimated by reviewer)
LOAEL (LOEL)	LOAEL= 4869ppm (estimated by reviewer) based on clinical observations, reduced wt gain.
Remarks	Two rats (1M, 1F) from group 3 (9137ppm) died on day 2; one female from group 3 died during exposure on day 1. Rats in groups 2 and 3 showed ocular discharge throughout d1-5. Rats in group 2 showed increased respiratory rate and dry red material around nose and mouth. All rats in group 2 were lethargic and showed labored respiration. Many rats in group 3 were lethargic and exhibited twitching and harsh respiratory sounds during days 1-5. All rats in group 2 and all but one survivor in group 3 appeared normal on day 8. Group mean body wt was significantly decreased in a dose related manner. No test article related effects were seen at gross necropsy on day 8; the male rat that died during the study showed gas in the G.I. tract and red-tinged fluid in the stomach.
Conclusions (study authors)	Exposure to test article caused a significant decrease in group mean body wt of male and female rats of low and high dose groups that was correlated with exposure level. Three deaths occurred in the high dose group during exposure. Major clinical signs were lethargy, twitching, harsh respiratory sounds and ocular discharge. No gross alterations were found in rats surviving to sacrifice.
Quality Reliabilities	**1.** Reliable without restrictions
References	Rausina, G.A. 1984. Five-day repeated dose inhalation toxicity study in rats of Hydrogenated Pyrolysis Gasoline. Proj. #2099. Gulf Life Sciences Center, Pittsburgh, PA
Other Last changed	Revised 7/27/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 5: High Benzene Naphthas

Acute Toxicity

Test Substance	Pyrolysis gasoline (Rerun Tower Overheads). Yellow, homogeneous liquid; 40% benzene, 26% C5, 13% toluene, 20% other. Test substance is described as a sample of a Pyrolysis Gasoline distillate fraction and is similar to Pyrolysis gasoline, a stream included in the test plan.
Method	
Method/guideline followed	Not specified
Type (test type)	Acute, limit test
GLP	Yes
Year	1994
Species/Strain	Rat, Sprague-Dawley
Sex	Males and females
No. of animals per sex per dose	5
Vehicle	None
Route of administration	Oral gavage
Test Conditions	Sprague Dawley rats (180-350g) were individually housed in stainless steel suspended cages and fasted overnight prior to administration of 2g/kg neat pyrolysis gasoline. The study room was maintained at 68-72^{0}F with a relative humidity of 35-63% and a 12 hr light-dark cycle. Water and chow diet were available ad lib after dosing. Test article was administered once on day 1 by oral gavage through a blunted needle. Rats were observed for clinical signs approx. 30 min, 1hr and 4hr, after dosing, and daily thereafter until sacrifice on day 15. Rats were checked once a day for mortality and moribundity. Observations were not made on weekends. Body wts were recorded prior to fasting and on days 1, 8 and 15.
Results	
LD_{50} with confidence limits.	The LD_{50} was not reached at 2g/kg. There were no deaths and all rats gained some weight during the study. Clinical signs noted in one or more rats were salivation, decreased activity, rales, lacrimation, chromodacryorrhea, ataxia, chromorhinorrhea, miosis, slight tremors, mydriasis , hyperactivity, hypothermia, urogenital discharge, nasal discharge, decreased food consumption, decreased fecal output, vocalization, and penile discharge. No gross pathological findings were noted at necropsy.
Remarks	
Conclusions (study author)	The LD_{50} was not reached at 2g/kg.
Data Quality	
Reliability	**1.** Reliable without restriction.
References	Rodriguez, S.C. and Dalbey, W.E. 1994. Acute oral toxicity of pyrolysis gasoline in Sprague Dawley Rats. Study #65636. Stonybrook Laboratories, Princeton, NJ. for Mobil Chemical Co., Edison, NJ.
Other	
Last changed	10/16/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 5: High Benzene Naphthas

Acute Toxicity

Test Substance	Pyrolysis gasoline (Rerun Tower Overheads). Yellow, homogeneous liquid; 40% benzene, 26% C5, 13% toluene, 20% other. Test substance is described as a sample of a Pyrolysis Gasoline distillate fraction and is similar to Pyrolysis gasoline, a stream included in the test plan.
Method	
Method/guideline followed	Not specified
Type (test type)	Acute, limit test
GLP	Yes
Year	1994
Species/Strain	Rabbit, New Zealand White
Sex	Males and females
No. of animals per sex per dose	3
Vehicle	None
Route of administration	dermal
Test Conditions	Rabbits, weighing at least 2kg, were individually housed in stainless steel suspended cages in a study room maintained at 69-72^{0}F with a relative humidity of 38-85% and a 12 hr light-dark cycle. Water and chow diet were available ad lib. The dorsal skin surface extending down from the front to rear legs and from left to right lower flanks was clipped free of hair the day prior to test article administration. Test article was spread evenly over the clipped area (approx. 10% of body surface area) at a dose of 2g/kg. A layer of 8-ply gauze was placed on the dorsal site, and a rubber dam sleeve was fitted snugly over the gauze pad and around the trunk. Edges of the dam were taped in place. An Elizabethan collar was affixed to the neck to prevent oral ingestion of test article and mechanical irritation of the test site. After 24 hrs, the collar and wrappings were removed and residual test article was wiped off. Body wts were recorded on days 1, 8 and 15. Rabbits were observed for toxicity at about 1 and 2 hr post-dose and daily thereafter on weekdays through day 14. Observations for mortality/moribundity were made daily. Rabbits were sacrificed on day 15 and necropsies were performed.
Results	
LD_{50} with confidence limits.	The LD_{50} was not reached at 2g/kg. There were no deaths during the study and rabbits either gained some weight or remained at day 1 body wt. Signs that might have resulted from treatment in one or more rabbits were: soft stool, decreased fecal pellet size, nasal discharge, and test site erythema. No gross pathological findings were noted at necropsy.
Remarks	
Conclusions (study author)	The LD_{50} was not reached at 2g/kg.
Data Quality Reliability	**1.** Reliable without restriction.
References	Rodriguez, S.C. and Dalbey, W.E. 1994. Dermal toxicity of pyrolysis gasoline in the New Zealand White rabbit. Study #65637. Stonybrook Laboratories, Princeton, NJ. for Mobil Chemical Co., Edison, NJ.
Other	
Last changed	10/16/2001 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 5: High Benzene Naphthas

Genetic Toxicity - in Vitro

Test Substance	Rerun Tower Overheads from Olefins/Aromatics Plant (light thermal cracked naphtha)
Test substance	CAS # 64741-74-8. Straw colored liquid; 40% benzene, 26% C5, 13% toluene, 20% other. Test substance is described as a sample of a Pyrolysis Gasoline distillate fraction and is similar to Pyrolysis gasoline, a stream included in the test plan.
Method	
Method/guideline followed	Standard method based on Ames et al, 1975
Type	Reverse mutation bacterial assay
System of testing	Salmonella typhimurium with and without metabolic activation
GLP	Yes
Year	1981
Species/Strain	S. typhimurium TA 98, TA100, TA1535, TA1537, and TA1538.
Metabolic activation	Yes
Species and cell type	Sprague Dawley male rat liver (S9 fraction) from Litton Bionetics, Kensington, MD
Quantity	50ul S9 fraction in 0.5ml S9 mix/plate
Induced or not induced	Aroclor 1254-induced, rats were given a single ip 500mg/kg dose, 5 days prior to sacrifice.
Concentrations tested	0, 0.029, 0.094, 0.30, 0.97µl/plate –S9, and 0.094, 0.30, 0.97, and 3.1µl/plate + S9; samples diluted in dimethyl sulfoxide (DMSO). Negative control 50µl DMSO
Statistical Method	None. Criteria for a positive response were an increase in revertant colonies at least two-fold that of negative control at the lowest active dose, and a dose response curve. Positive results must be reproducible in an independent repeat assay.
Remarks for Test Conditions	Rerun tower overheads test solutions were prepared in DMSO immediately prior to use. Salmonella (Approx. 1.4-2x10^8 cells/ml) were exposed to either test solution or DMSO ±S9 by the preincubation method. Doses of 0.029-0.97µl/plate-S9 and 0.094-3.1µl/plate +S9 were determined by a pretest toxicity test in TA 100 and TA1537±S9 using incremental doses from 0.01-10µl/plate. Culture tubes containing 50µl test solution or DMSO, 0.1ml Salmonella and 0.5 ml phosphate buffer or S9 mix were combined and incubated with shaking (150 rpm) for 20 minutes at 37^0C. At the end of the preincubation period, top agar was added, mixed and cultures were overlaid on minimal agar plates, 3 plates/dose/strain. Plates were incubated at 37^0C for 48 hrs, then counted automatically (Biotran II) and background lawn evaluated by stereomicroscope. Positive control compounds were: -S9, 2-nitrofluorene (2-NF, 20µg/plate) for TA98 and TA1538; N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 2.0µg/plate) for TA100 and TA1535; 9-aminoacridine (9-AA, 25µg/plate) for TA1537; +S9 2-aminoanthracene (2µg/plate) for all strains except TA1537.
Results	
Genotoxic effects	The preliminary toxicity test exhibited severe toxicity at 10µl/plate with activation and at 3.1 and 10µl/plate without activation (individual data not shown). In the mutagenicity test, none of the 5 strains of Salmonella exhibited revertant frequencies substantially different from the solvent or spontaneous controls at any dose level with or without metabolic activation (e.g. TA98-S9: 16, 15, 12, 12, and 0 average revertants/plate and TA100-S9: 111, 115, 107, 94, and 0 at 0[DMSO], 0.029, 0.094, 0.30, and 0.97µl/plate, respectively: TA98+S9: 33, 26, 26, 22, and 0 revertants/plate, and TA100+S9: 128, 161, 128, 118, and 0 revertants/plate at 0[DMSO], 0.094, 0.30, 0.97 and 3.1µl/plate, respectively). Clearing of background lawn and microcolonies were observed at the maximum doses (0.97µl/plate-S9; 3.1µl/plate+S9). Positive control compounds (2 plates/strain) performed appropriately (-S9: MNNG 1906, 1883 revertants/plate in TA 100 and TA1535, respectively; 9-AA 586 revertants/plate in TA1537; 2-NF 2114, 1214 revertants/plate in TA98 and TA1538, respectively; and +S9 2-aminoanthracene 406-2307 revertants/plate for all strains except TA1537). The results of this assay indicate that rerun tower overheads had no mutagenic activity in this test system. (Reviewer's note: Due to toxicity, tests were performed over a low dose range; 3 of 4 doses were non-toxic and showed sufficient growth to evaluate mutagenicity. Testing at any lower doses was impractical).

Conclusions	Rerun Tower Overheads did not induce an increase in revertant colonies in any Salmonella
(contractor)	strain, tested at any dose level with or without metabolic activation in this single Ames test.
Data Quality	
Reliabilities	**1.** Reliable without restriction
Reference	Blackburn, G.R. 1981. An Ames Salmonella/mammalian microsome mutagenesis assay for the determination of potential mutagenicity of Rerun Tower Overheads from an olefins/aromatics plant. Study No. 1781-80. Mobil Environmental and Health Sciences Laboratory, Princeton, NJ. Ames B. N. et al. 1975. Mutat. Res. 31: 347-364.
Other	
Last changed	10/02/2001 (Prepared by a contractor for the Olefins Panel)

Robust Summary - Group 5: High Benzene Naphthas

Genetic Toxicity - in Vitro

Test Substance	Rerun Tower Overheads from Olefins/Aromatics Plant (light thermal cracked naphtha)
Test substance	CAS # 64741-74-8. Straw colored liquid; 40% benzene, 26% C5, 13% toluene, 20% other. Test substance is described as a sample of a Pyrolysis Gasoline distillate fraction and is similar to Pyrolysis gasoline, a stream included in the test plan.
Method	
Method/guideline followed	None specified. Standard method based on Slater et al., 1971, Green and Muriel, 1976, and Ames et al., 1973.
Type	Bacterial DNA repair
System of testing	*Escherichia coil, Salmonella typhimurium*
GLP	Not specified
Year	1978
Species/Strain	*E. coli* WP2 uvrA$^+$ recA$^+$, WP100 uvrA$^-$ recA$^-$; *S. typh*. TA1978 uvrB$^+$, TA 1538 uvrB$^-$
Metabolic activation	Yes
Species and cell type	Sprague Dawley male rat liver (S9 fraction)
Quantity	50µl S9 fraction in 1.0ml S9 mix/plate
Induced or not induced	Aroclor 1254 induced (single ip injection of 500mg/kg, 5 days prior to sacrifice)
Concentrations tested	Spot test: 10µl/plate undiluted
Statistical Methods	None. Compounds that cause damage to DNA will more severely affect repair deficient strains than repair proficient stains. Toxic compounds that do not affect DNA will not induce differential toxicity.
Remarks for Test Conditions	Tester strains were stored in liquid nitrogen and fresh cultures were inoculated directly from frozen stock, grown overnight at 37^0C, re-diluted and grown to final cell concentration of $2x10^8$ cells/ml. Each test article -strain combination was plated in triplicate with and without metabolic activation. Log phase cultures (0.1ml) added to 2.5ml top agar were poured on Vogel-Bonner minimal medium plates. For plates without activation, a 6.5mm paper disc (antibiotic type) was placed in the center of each plate; 10µl test article is placed on disc. For plates with S9 activation, after top agar sets, a 9.5mm diameter hole was cut in agar in the center of the plate, the well was sealed with 0.1ml top agar, and 150µl of S9 mix/control or test article mix (14:1) added to the well. All inverted plates were incubated at 37^0C for 24hr. The diameter of any resulting zone of inhibition was measured in mm. Zone diameter of a repair deficient strain was divided by the zone diameter of the repair proficient parent strain. Positive control compounds were 4-nitro-quinoline-1-oxide (4-NQO; 30µg/plate) –S9, 2-aminofluorene (2-AF; 250µg/plate) +S9, and negative control was 25µg/plate penicillin. Tests were performed twice ± S9.
Results	
Genotoxic effects	In duplicate tests, average inhibition ratios induced by Rerun tower overheads –S9 were 1.4, 1.8 for *E. coli* WP100/WP2, and 1.3, 1.5 for *S. typh*. TA1538/TA1978 compared to negative control values of 1.0, 1.1, and 1.1, 1.2 in *E coli* strains and *S. typh*. strains, respectively, suggesting a weak differential killing of repair deficient strains without metabolic activation. Positive control ratios for 4-NQ –S9 were 2.3, 2.5 for *E coli* WP100/WP2, and 1.7, 1.6 for *S. typh*. TA1538/TA1978. In tests with metabolic activation (+S9), average inhibition ratios were 1.0, 1.0 for *E.coli* strains and 1.0, 1.0 for *S. typh*. strains in duplicate tests compared to negative control values of 1.1, 1.1, and 1.1, 1.1 in *E. coli* and *S. typh*. strains, respectively, indicating no test article induced toxicity. Positive control, 2-AF, inhibition ratios were 2.1, 2.1 for *E. coli* WP100/WP2, and 1.9, 1.4 for *S. typh*. TA1538/TA1978.
Conclusions (contractor)	Rerun tower overheads did cause weak differential killing in DNA repair deficient strains, *E. coli* WP100 and *S. typhimurium*. TA1538 in the absence of metabolic activation, suggesting that the test article can cause direct acting damage to bacterial DNA. No differential killing was observed in the presence of metabolic activation.
Data Quality	
Reliabilities	**1.** Reliable without restriction

Reference	Haworth, S.R. 1978. Bacterial DNA repair assay of Mobil Chemical Company Compound MCTR-125-78 (MRI #110). E. G. and G. Mason Research Institute, Rockville, MD. for Mobil Chemical Co, Edison, NJ Slater, E.E. et al. 1971. Cancer Res. 31: 970-973. Green, M.H.L. and Muriel, W.J. 1976. Mutat. Res. 38:3-32 Ames, B.N. et al. 1973. Proc. Natl. Acad. Sci., USA 70: 782-786.	
Other *Last changed*	2/28/2002 (Prepared by a contractor to the Olefins Panel)	

Genetic Toxicity - in Vitro

Test Substance	Rerun Tower Overheads from Olefins/Aromatics Plant (light thermal cracked naphtha)
Test substance	CAS # 64741-74-8. Straw colored liquid; 40% benzene, 26% C5, 13% toluene, 20% other. Test substance is described as a sample of a Pyrolysis Gasoline distillate fraction and is similar to Pyrolysis gasoline, a stream included in the test plan.
Method	Standard method, no guideline specified
Method/guideline followed	Cell transformation
Type	Mouse embryo cells
System of testing	Yes
GLP	1981
Year	BALB-c/3T3 mouse cell line
Species/Strain	No
Metabolic activation	NA
Species and cell type	NA
Quantity	NA
Induced or not induced	Initial cytotoxicity: 0, 0.01, 0.1, 1.0, 10.0, 100.0µg/ml medium; Transformation: 0. 0.8, 4.0, 20.0 and 100µg/ml, diluted in dimethyl sulfoxide. Negative control was DMSO at 2.5% vol. concentration.
Concentrations tested	T-test specified. Standard criteria for positive response is a two fold increase in type III foci at highest dose over vehicle control with or without a dose related response or a 2 fold increase at 2 or more consecutive doses.
Statistical Method	Routine procedures were referred to Appendix 1 Standard Operating Procedures, which was not included with this report. Only specifics unique to this assay are presented. Due to the volatile nature of test material, the cytotoxicity assay and transformation assays were conducted in tightly capped T-25 flasks in sealed plastic bags. The pH of medium during the 72hr exposure period was maintained at 7.4 by 0.02M Hepes buffer in flasks. RTO was prepared as a 1% stock solution in DMSO, which, when added to culture medium at a 2.5% vol. conc. was a suspension. Despite limited solubility, RTO produced a dose-dependent cytotoxic effect after a 3-day exposure period. In the initial toxicity assay, RTO was added to flasks, seeded with BALB-c/3T3 cells, at concentrations of 0, 0.01, 0.1, 1.0, 10.0 and 100.0µg/ml, incubated for 3 days at 37^0C in a CO_2 in air incubator, after which cells were counted for survival. In the transformation assay, RTO was tested at 0, 0.8, 4.0, 20.0 and 100µg/ml. In a standard BALB-c/3T3 transformation assay, colony formation cultures (approx. 100 cells/culture) and transformation cultures (approx. 10^4 cells/culture, 20 cultures/dose) were seeded on day 1, exposed to test material for 2-3 days, and culture medium was changed on day 4. For transformation cultures, medium continued to be changed weekly to day 29. Colony formation cultures were fixed, stained and counted visually on day 8 to determine cloning efficiency; transformation cultures were fixed and stained on day 29 for focus counting and evaluation. Transformation frequency = total type III foci ÷ total cultures/dose. Positive control compound was 3-methyl cholanthrene (2µg/ml).
Results	RTO induced toxicity in BALB-c/3T3 cells after 3 days exposure at concentrations of 10µg/ml (59% viability) and at 100µg/ml (18% viability). In the transformation assay, inhibition of cloning efficiency (CE, clones/100 cells) occurred at 4.0µg/ml (89% CE), 20.0µg/ml (81% CE) and 100µg/ml (65% C.E.); cell toxicity was somewhat less than in the initial cytotoxicity assay [40% viability at 100µg/ml]. RTO did not induce statistically significant increased incidence of transformed foci compared to negative controls at any dose level. Values were 0.10 foci/flask, 2/20 flasks with foci at 100µg/ml, 0.0 foci/flask, 0/20 flasks with foci at 20.0µg/ml, 0.15 foci/flask, 3/20 flasks with foci at 4.0µg/ml, 0.10 foci /flask, 2/20 flasks with foci at 0.8µg/ml compared to 0.05 foci/flask, 1/20 flasks with foci in negative control group. [Reviewer's note: Negative control value of 1 focus/20 flasks was lower than control values in other concurrent studies on 2 other compounds in this series where negative controls had 4 foci in 20 flasks (0.20 foci/flask)]. Positive
Genotoxic effects	

	control compound, 3 methyl cholanthrene, induced 56 foci/19 flasks (2.95 foci/flask), 18/19 flasks with foci.
Conclusions (contractor)	Rerun tower overheads did not induce neoplastic transformation in BALB-c/3T3 cells and was not active in this test system.
Data Quality *Reliabilities*	**2.** Reliable with restrictions. Complete details of assay methods are not included in the report. Specifics of statistics are not supplied.
Reference	Tu, A.S. and Sivak, A. 1981. BALB-c/3T3 Neoplastic transformation assay on 0818802, 08188003 and 08188005 (Rerun tower overheads). ALD Ref. #86374. Arthur D. Little, Inc. Cambridge, MA for Mobil Oil Corp, Study #1771-80, Princeton, NJ Roy, T.A., 1981. Analysis of rerun tower bottom oil by combined capillary gas chromatography/mass spectrometry. Study #1272-81-. Toxicology division, Mobil Oil Co., Princeton, NJ
Other *Last changed*	12/07/01 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vitro

Test Substance	Rerun Tower Overheads from Olefins/Aromatics Plant (light thermal cracked naphtha)
Test substance	CAS # 64741-74-8. Straw colored liquid; 40% benzene, 26% C5, 13% toluene, 20% other. Test substance is described as a sample of a Pyrolysis Gasoline distillate fraction and is similar to Pyrolysis gasoline, a stream included in the test plan.
Method	
Method/guideline followed	None specified. Standard method based on Clive and Spector, 1975
Type	Mammalian cell mutation assay
System of testing	Mouse lymphoma cells
GLP	Not specified
Year	1979
Species/Strain	Mouse lymphoma L5178Y TK+/- cells
Metabolic activation	Yes
Species and cell type	Sprague Dawley males rat liver (S9 fraction)
Quantity	50µl S9 fraction/ml S9mix)
Induced or not induced	Aroclor 1254 induced (single ip injection of 500mg/kg, 5 days prior to sacrifice)
Concentrations tested	-S9 cloned doses: 0.0, 0.013, 0.018, 0.024, 0.032, 0.042, 0.056, 0.075, and 0.10µl/ml +S9 cloned doses: 0.0, 0.048, 0.063, 0.085, 0. 11, 0.15, 0.20, 0.27, and 0.36µl/ml. All doses diluted in acetone
Statistical Methods	None. Compound was designated as mutagenic if it induced a mutation frequency (mutant cells/10^4 surviving cells) greater than 3 times the standard error (S.E. [f]) calculated by formula from the viable counts and total mutant cells (trifluorothymidine resistant cells) at each dose level.
Remarks for Test Conditions	Freshly prepared actively growing cultures of L5178Y cells ($1x10^6$ cells/ml) were dispensed in 6ml aliquots into 44 polypropylene centrifuge tubes. Rerun tower overheads, solubilized in acetone, beginning at a concentration equal to LD90 from a preliminary toxicity test, was diluted over 15 serial 1/8 log dilutions, producing 16 dose levels decreasing approximately 100 fold from highest to lowest, and added to cells in the centrifuge tubes. Four ml of S9 activation mixture or 4ml culture medium was added, yielding a final cell suspension of $0.6x10^6$ cells/ml. Positive control compounds were ethyl methyl sulfonate (EMS, 1.0µl/ml) –S9 and 7,12-dimethylbenzanthracene (7,12-DMBA, 2.5µl/ml) +S9 cultures. All tubes were gassed with 5% CO2/air and placed on a roller drum for 4hrs at 37^0C in the dark. At the end of exposure, calls were washed with fresh medium, re-suspended, gassed, replaced on roller drum at 37^0C and incubated for 3 days with a cell population adjustment every 24 hrs to maintain a cell population density of $0.3x10^6$ cells/ml. After 3 days expression, 8 cultures ± S9, which exhibited toxicity from 10-90% growth inhibition during the expression period, were selected for cloning. At cloning, cells were placed in restrictive suspension medium containing trifluorothymidine (TFT, 1µg/ml) that allows only TK-/- cells to grow. Two Florence flasks/concentration ± S9, one for restrictive medium, on for viable cell counts, were filled with 100ml cloning medium and maintained at 37^0C. Six 100mm petri plates/concentration ± S9 were prepared, 3 for restrictive medium, 3 for viable cell counts. Cell counts were made from each centrifuge tube to determine the volume of cell population = $3x10^6$cells. This volume was retained, centrifuged and the supernatant discarded except for 2ml in which cells were re-suspended and placed in restrictive medium flask. A $5x10^{-4}$ dilution was prepared and added to the appropriate viable count flask containing 100ml cloning medium. After this dilution, 1 ml of TFT stock solution was added to the restrictive medium flask and incubated with shaking (125rpm) at 37^0C for 15min. Flasks were removed, 33ml of cell suspension was pipetted into each of 3 appropriately labeled plates and placed in the cold (4^0C) for 20 min to accelerate gelling. Plates were removed and incubated at 37^0C in humidified 5% CO2/air for 10 days. At the end of incubation, plates were scored for total number of colonies/plate, 3 counts/plate, on an automated colony counter. Mutation frequency (MF) = avg. number of colonies in 3 restrictive medium plates ÷ avg. number of colonies $x10^4$ in 3 corresponding viable count plates. Induced mutation frequency (IMF) = MF test article – MF solvent control.

Results Genotoxic effects	In cultures without metabolic activation, the two highest concentrations cloned, 0.10µl/ml (MF=1.4, IMF=0.8) and 0.075µl/ml (MF=1.0, IMF=0.4) exhibited slight dose related increases in IMF compared with acetone control (MF=0.6); only the 0.10µl/ml concentration caused a doubling of MF over controls. EMS positive control values were MF=27.1, IMF=26. The first activated assay was discarded due to loss of positive control cultures by contamination. In the repeat test with metabolic activation, 2 dose concentrations had MF 2 times greater than acetone controls: the highest dose cloned, 0.36µl/ml (MF=0.8, IMF=0.4) and 0.15µl/ml, the 4th highest dose cloned (MF=0.9, IMF=0.5) versus control (MF=0.4). However, intervening cloned doses of 0.20, and 0.27µl/ml did not show increased MF; the values for the positive doses were not dose related and were within the range of experimental error for the assay. Positive control values +S9 for 7,12- DMBA were MF=2.6, IMF=2.0.
Conclusions (contractor)	Without metabolic activation, Rerun tower overheads appears to induce a weak mutagenic response at the two highest doses only; a dose response trend was not observed in the 6 lower doses cloned. Test article did not induce significant mutagenic activity in cultures containing S9, suggesting that any mutagenic activity is suppressed or inactivated by the presence of the liver microsome metabolizing system.
Data Quality *Reliabilities*	**1.** Reliable without restriction.
Reference	Kirby, P.E. et al., 1979. An evaluation of mutagenic potential of MCTR-125-78 (MRI #110) employing the L5178Y TK+/- mouse lymphoma assay. E.G. and G. Mason Research Institute, Rockville, MD for Mobil Chemical Co., Edison, NJ Clive, D., and Spector, J.F.S. 1975. Mutat. Res. 31: 17-29
Other *Last changed*	2/28/2002 (Prepared by a contractor to the Olefins Panel)

Robust Summary - Group 5: High Benzene Naphthas

Genetic Toxicity - in Vitro

Test Substance	Rerun Tower Overheads from Olefins/Aromatics Plant (light thermal cracked naphtha)
Test substance	CAS # 64741-74-8. Straw colored liquid; 40% benzene, 26% C5, 13% toluene, 20% other. Test substance is described as a sample of a Pyrolysis Gasoline distillate fraction and is similar to Pyrolysis gasoline, a stream included in the test plan.
Method	
Method/guideline followed	None specified. Standard method based on Bertram, 1977
Type	Mammalian cell transformation assay
System of testing	Mouse C3H embryo cells
GLP	Not specified
Year	1978
Species/Strain	Mouse embryo cells/ C3H 10T½
Metabolic activation	No
Species and cell type	NA
Quantity	NA
Induced or not induced	NA
Concentrations tested	0, 0.625, 1.25, 2.5 and 5.0µl/ml, all diluted in acetone
Statistical Methods	None. A positive response is determined by the appearance of any type II foci (50% can be malignantly transformed) and type III foci (85% can be malignantly transformed) compared to negative controls. The C3H 10T½ cell line has no spontaneous transformation.
Remarks for Test Conditions	For the preliminary toxicity assay, cells (200/plate) were exposed to Rerun tower overheads diluted in acetone, over a range of concentrations from 0.0003-5.0µl/ml, at 2-fold dilutions for 18hrs; cells were then washed, re-fed with fresh Eagle's basal medium and incubated for 10 days in 5% CO2/air at 37^0C. After incubation, cells were washed, fixed with absolute methanol (20 min) and stained with Giemsa (30 min); number of cells/plate were counted and cloning efficiency (CE) determined=Avg. number colonies/plate ÷ number cells plated x100. In the transformation assay, cells in late log phase were plated at a concentration of $1x10^3$ cells/60mm petri dish. Cultures for concurrent toxicity determination were prepared at 200 cells/plate. After 24 hrs, cultures were treated with appropriate test article concentrations in 25µl volumes at 4 dose levels, 12 plates/dose, in decreasing 2-fold dilutions from concentrations which exhibit 25-75% relative CE. Positive control compound was 7, 12-dimethylbenzanthracene (7, 12-DMBA, 0.5µg/ml). After 18hr treatment, test article was removed, cultures were re-fed, and re-incubated. Toxicity plates were incubated for 10 days, stained and CE determined. Transformation cultures were re-fed weekly until 35 days after removal of test article had elapsed. All plate cultures were washed, fixed, stained and scored for the presence of type II and type III foci by macroscopic and microscopic examination. Type II foci show massive piling up in virtually opaque monolayers, cells are moderately polar. Type III foci are composed of highly polar, fibroblastic, multilayered, criss-crossed arrays of densely stained cells.
Results	
Genotoxic effects	Rerun tower overheads induced 71% relative cloning efficiency at 5.0µl/ml; transformation assay was performed at 2-fold dilutions from 5.0µl/ml. In the toxicity study conducted in parallel with the transformation assay, test article induced 100% cell death at 5.0µl/ml. In the transformation assay, sufficient cells survived to form a confluent layer in 8/12 plates at 5.0µl/ml dose level after 35 days. No indication of type II or type III foci were induced by rerun tower overheads at any dose level. Positive control, 7,12-DMBA induced 9 type II and 12 type III foci on 12 plates.
Conclusions	
(contractor)	Rerun tower overheads does not induce cell transformation in mouse embryo C3H 10T½ cells.
Data Quality	
Reliabilities	**1.** Reliable without restriction

Reference	Jensen, E.M., and Thilager, A.K. 1978. C3H 10T½ cell transformation assay, Mobil Chemical Co. Compound MCTR-125-78 (MRI #110). E.G. and G. Mason Research Institute, Rockville, MD Bertram, J.S. 1977. Cancer Res. 37: 514-523
Other *Last changed*	2/28/2002 (Prepared by a contractor to the Olefins Panel)

Genetic Toxicity - in Vivo

Test Substance	
Remarks	Rerun Tower Overheads from Olefins/Aromatics Plant (light thermal cracked naphtha) CAS # 64741-74-8. Straw colored liquid; 40% benzene, 26% C5, 13% toluene, 20% other. Test substance is described as a sample of a Pyrolysis Gasoline distillate fraction and is similar to Pyrolysis gasoline, a stream included in the test plan.
Method	
Method/guideline followed	None specified. Standard method based on Bowman, 1969; Lewis, 1954; Mendelson, 1976
Type	*Drosophila* assays for point mutation, chromosome aberrations & chromosome loss
GLP	Not specified
Year	1979
Species	*Drosophila melanogaster*
Strain	Dominant lethal: Canton S; Y chromosome loss: males red/white eye; females white/ white eye; Somatic reversion: males white ivory ($\underline{w^i}$), yellow body ($\underline{y}$), echinus ($\underline{ec}$); females $\underline{w^i}/\underline{w^i}$; Bithrox test: males Ultrabithorax ($\underline{Ubx}$); females bithorax ($\underline{bx^{34e}}$); Sex-linked recessive lethal: males Canton S; females $\underline{Basc}/\underline{Basc}$
Sex	Males and females
Route of administration	Aerosol
Doses/concentration levels	0.3ml in 50ml air
Exposure period	10 min.
Statistical methods	Events in these tests have very low probabilities. Analysis based on Poisson distribution with fiducial limits computed according to Stevens, 1942.
Remarks for Test Conditions.	*Drosophila* stocks were maintained in agar/corn meal/sugar/yeast medium at 23^0C. One set of stocks was transferred each week to isolate virgin females for breeding. Four days are required for maturation of *Drosophila* sperm cells after meiosis. In all assays, treated males were mated for 3 days only to assure use of a uniform sample of treated sperm. In all assays, test article was administered as an aerosol, 0.3ml in 50ml volume of air.when administered for 1hr anesthesized flies and killed approximately 30%. Longer treatments reduced fertility. Exposure in all assays was 10 minutes in duration. Somatic reversion of white-ivory: Larvae from mating of males carrying 5 copies of white-ivory gene on the X chromosome ($\underline{w^i}$, $\underline{y}$, $\underline{ec}$) with $\underline{w^i}/\underline{w^i}$ females were treated with aerosolized test article for 10 min. Positive control compound was 0.04M mitomycin C. Larvae were washed and transferred to culture bottles to complete development. After eclosion, female offspring, genotype Qn(1)$\underline{w^i}$, $\underline{y}$, $\underline{ec}/\underline{w^i}$ were scored for red spots in the eye, which signals reversion of $\underline{w^i}$ to a pigment cell. Y chromosome loss: Males carrying a duplication of the gene for normal (red) eyes on Y chromosome and a mutant allele, white ($\underline{w}$) on the X chromosome were treated with aerosolized test article for 10 min and mated to white-eyed females ($\underline{w}/\underline{w}$). Positive control were males exposed to 3kr X-rays. Frequency of occurrence of white-eyed male progeny measured frequency of Y chromosome loss. Dominant lethal mutations: Defined as any genetic change that blocks development prior to hatching. Treated Canton S males were mated with untreated females in nylon net cages on Welch's grape juice solidified with 2% agar. After 12 hr, agar plates were removed and stored at room temp. (23^0C) for 30 hrs. Positive control was 0.04M ethyl methane sulfonate. Eggs were scored for hatching after 30hrs. Bithorax test of Lewis: Occurrence of rearrangements with one breakpoint between centromere and the locus of bithorax ($\underline{bx}$) was determined by scoring offspring of treated Ultrabithorax males and bithorax females. Males treated with 3kr X-rays were the positive controls. Distinctive phenotype was the presence of a mesonotum Sex-linked recessive lethals: Canton-S males, treated with test article, were mated with Basc (balancer X chromosome) females. Individual (F1) female progeny were mated with Basc males. Any single female culture containing at least 20 flies (F2), at least 8 of which are males, but no males are wild type, is scored as a lethal. Ethyl methane sulfonate (0.04M) treated males were the positive controls. A repeat study was performed due to loss of cultures to dessication.

Results Genotoxic effects NOAEL (NOEL) LOAEL (LOEL)	Rerun tower overheads did not induce genetic damage in *Drosophila melanogaster* under experimental conditions in any test employed. The repeated sex-linked recessive lethal test, performed due to technical problems in the initial assay, did not demonstrate any genetic damage in *Drosophila* from exposure to the test article
Conclusions (study authors)	Rerun tower overheads did not induce genetic damage in *Drosophila melanogaster*.
Data Quality *Reliabilities*	**1.** Reliable without restrictions.
References	Bowman, J.T. 1979. *Drosophila* mutagenicity assays of Mobil Chemical Compound MCTR-125-78. MRI #110. E.G. and G. Mason Research Institute, Rockville, MD, for Mobil Chemical Co., Edison, NJ. Bowman, J.T. 1969. Mutat. Res. 7: 409-415 Lewis, E.B. 1954. Am. Nat. 88: 225-239 Mendelson, D. 1976. Mutat. Res. 41: 269-276 Stevens, W.L. 1942. J. Genetics 43: 301-307
Other *Last changed*	2/28/2002 (Prepared by a contractor to the Olefins Panel)

Repeated Dose Toxicity

Test Substance	Rerun Tower Overheads from Olefins/Aromatics Plant (light thermal cracked naphtha)
Remarks	CAS # 64741-74-8. Straw colored liquid; 40% benzene, 26% C5, 13% toluene, 20% other. Test substance is described as a sample of a Pyrolysis Gasoline distillate fraction and is similar to Pyrolysis gasoline, a stream included in the test plan.
Method	
Method/guideline followed	None specified, comparable to standard methods
Test type	Subacute
GLP	Not specified
Year	1980
Species	Rabbit (4/sex/group)
Strain	New Zealand White
Route of administration	Dermal
Duration of test	21 days
Doses/concentration levels	0, 0.1, 0.5, and 1.0ml/kg/day
Sex	Male and females
Exposure period	Continuous (no wipe-off)
Frequency of treatment	Once/day
Control group and treatment	Males and females (4M, 4F), saline (0.9%), 1ml/kg/day
Post exposure observation period	3 days
Statistical methods	Bartletts test, analysis of variance, Scheffe's multiple pair wise comparison, Gaines and Howell's multiple pair wise comparison
Test Conditions	Rabbits were housed individually in stainless steel cages and received water and rabbit chow diet, ad lib. Initial body wt ranged from 2455-3005g for males and 2455-3035g for females. Four rabbits of each sex were assigned to treatment groups of 0, 0.1, 0.5, and 1.0ml of neat test article/kg/day. Control rabbits received 1.0ml/kg/day of 0.9%NaCl. Prior to initiation, the dorsal dosing area was clipped free of hair and clipping was done periodically during the study. The exposure area was abraded with minor incisions deep enough to penetrate the stratum corneum but not deep enough to produce bleeding. Abrasions were made prior to the first application, and thereafter, on the first day of each week. Test article was applied to the skin once a day, starting on day 1, for 21 consecutive days; rabbits were sacrificed between day 22 and day 24. Each rabbit wore a plexiglass collar for the entire study to retard ingestion of test article. Rabbits were observed daily for mortality and moribundity, food/water intake, general appearance/behavior, toxic/pharmacological effects, and dermal reactions for 24 consecutive days. Dermal irritation was graded each morning prior to dosing. Food consumption was determined 3 times /wk and body weight on days1, 8, 15, and at termination. Prior to study initiation and during wk 3, hematocrit (Hct), hemoglobin (Hgb), erythrocyte count (RBC), total leukocyte count (WBC) and differential leukocyte count, mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin conc. (MCHC), serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (Alk Phos), fasting glucose, and blood urea nitrogen (BUN); urine (pH, specific gravity, glucose, ketones, total protein, bilirubin[BIH]), and microscopic examination of sediment were evaluated. Rabbits were sacrificed on day 24; necropsies were performed and gross observations recorded for all rabbits. Liver, kidney, thyroid, and adrenals were weighed and preserved for microscopic analysis along with brain, pituitary, lung, heart, spleen, pancreas, urinary bladder, testis/ovary, skin, and any unusual lesions.
Results	
NOAEL (NOEL)	NOAEL <0.10ml/kg/day both sexes (skin irr). LOAEL = 0.1ml/kg/day both sexes (skin irr)
LOAEL (LOEL)	NOAEL = 1.0ml/kg/day both sexes (systemic effects). LOAEL >1.0ml/kg/day both sexes (systemic effects).
Remarks	Two rabbits died during the study from cardiac puncture blood sampling. No test article induced effects were noted during clinical observations. Two 0.1ml/kg/day group males and one female showed erythema from day 10 to termination; 3 0.5ml/kg/day group males showed erythema from day 8 to termination; all 0.5ml/kg/day males and females, and

	1.0ml/kg/day males and females had well defined erythema from day 9 to termination. Edema was not present in any rabbits. Skin thickening was noted in all test article groups from wk 1 to termination. Fissuring was seen in 3 0.1ml/kg/day males, 3 0.5ml/kg/day males and all 1.0ml/kg/day males; all test article treated females showed fissuring. Necrosis was present in 2males and 3 females given 0.1ml/kg/day, 3males and all females given 0.5ml/kg/day, and all males and females given 1.0ml/kg/day. There were no significant changes in body wt or food consumption between controls and treatment groups. Terminal basophilic values were elevated in all male test article treated groups; all other hematology values were comparable to controls. Urinalysis findings were unremarkable. There were no significant differences in organ wt between control and any treatment group. Histological evaluation for the skin showed effects consistent with gross observations with no dose-related gradation of severity between doses, including hyperkeratosis, acanthosis, accumulation of heterophils, and cellular debris in stratum corneum, and hyperplasia of sebaceous glands. There were no abnormal microscopic findings attributable to test article administration in organs from the three treatment levels compared to controls.
Conclusions (study authors)	Daily epidermal application of test article resulted in skin irritation at the application site.
Quality Reliabilities	**2.** Reliable with restriction. There was no mention of GLP.
References	Fieser, S., Alsaker, R.D., Brown, H.R., and Wolfe, G.W. 1980. 21-Day dermal irritation study in rabbits. Proj. #230-213. Hazleton Laboratories America, Inc., Vienna, VA. For Mobil Chemical Co., Edison NJ (This study was actually for subacute toxicity, not only skin irritation)
Other Last changed	2/28/2002 (Prepared by a contractor to the Olefins Panel)

Developmental Toxicity/Teratogenicity

Test Substance	
Remarks	Rerun Tower Overheads from Olefins/Aromatics Plant (light thermal cracked naphtha) CAS # 64741-74-8. Straw colored liquid; 40% benzene, 26% C5, 13% toluene, 20% other. Test substance is described as a sample of a Pyrolysis Gasoline distillate fraction and is similar to Pyrolysis gasoline, a stream included in the test plan.
Method	
Method/guideline followed	None specified, conforms to standard method
Test type	Teratology
GLP	Yes
Year	1981
Species	Rabbit
Strain	New Zealand White
Route of administration	Oral gavage
Concentration levels	0, 10, 25, and 50mg/kg/day, diluted in corn oil
Sex	Pregnant females (16/group)
Exposure period	Day 6-28 of gestation
Frequency of treatment	Once/day
Control group and treatment	16 pregnant females received 0.5ml/kg/day corn oil
Duration of test	29 days
Statistical methods	Chi square with Yates' correction for 2x2 contingency table and /or Fisher's exact probability test; Mann-Whitney U test; analysis of variance (one-way), Bartlett's test and t-test using Dunnett's multiple comparison tables. Level of significance $p<0.05$.
Remarks for Test Conditions.	In an initial study, RTO was administered by oral gavage, undiluted to 16 pregnant rabbits/group at levels of 0 (distilled water), 10, 25 and 50 mg/kg/day. Forty-two rabbits died: 14, 11, 13, and 13 in the 0, 10, 25 and 50mg/kg/day groups respectively. Due to excess mortality in all treated groups and the controls, the study was terminated and repeated at the same concentrations diluted in corn oil. Sixty-four sexually mature virgin female rabbits (7 months old, 3.46-4.19kg at study initiation) were acclimated for 59 days, assigned a unique animal number and ear-tagged when placed on study. All rabbits were individually housed in suspended wire cages and maintained in a temperature, humidity, and light (12 hr light/dark cycle) controlled environment. Certified rabbit chow and tap water were available ad lib. Only coccidiosis-free rabbits were used in the study. Prior to insemination, females were randomly assigned to groups (16/group) according to body wt, by a computer-generated program. Sperm was collected from each of 6 proven breeder males of the same source and strain, using an artificial vagina. Semen was immediately evaluated for motility, and was used for insemination only if motility was=50%. Useable ejaculate was diluted in 0.9%NaCl at 35^0C; 0.25-0.50ml of dilute semen was introduced into the anterior vagina. Ovulation was induced by injection of 100 units chorionic gonadotropin (Ayerst, NY) in the marginal ear vein of the female immediately after insemination. Semen from one male was used to inseminate an equal number of females in each group. Inseminations were performed on two consecutive days; an equal number of females was inseminated in each group/day, designated as day 0 of gestation. RTO was mixed with corn oil daily at appropriate doses and shaken by hand. No analysis of dosing solution was reported. Negative control dams were given 0.5ml corn oil/kg/day, the volume equal to the highest treatment group. Individual doses were determined from individual body wt on day 6 of gestation. Females were observed daily for mortality, overt changes in appearance and behavior, and, from day 6-29 of gestation, for clinical signs of toxicity. One dam aborted on gest. day 19 and remained on study until scheduled sacrifice; aborted material was discarded. Body wt was recorded on gestation days 0, 6, 12, 18, 24, and 29. On gest. day 29, all females were sacrificed by overdose of sodium pentabarbitol, uteri were excised and weighed prior to removal of fetuses. Number and location of viable and non-viable fetuses, early and late resorptions, number of total implantations, and corpora lutea were recorded. Abdominal and thoracic cavity and organs of dams were examined grossly and discarded. Uteri from apparently non-gravid animals were opened and placed in 10% ammonium sulfide solution to confirm pregnancy status.

	All fetuses were individually weighed and examined for external malformations and variations. Each fetus was internally sexed and examined for internal malformations and variations, including the brain by mid-coronal slice. The heart was dissected using Staples' technique. Eviscerated, skinned fetuses were individually numbered and tagged, fixed in alcohol, macerated and stained with Alizarin Red S for skeletal examination. Fetal findings were classified as malformations or genetic or developmental variations.
Results	
NOAEL maternal toxicity	NOAEL maternal = 25mg/kg/day (based on 1 abortion at 50mg/kg/day)
NOAEL developmental toxicity	NOAEL developmental = 50mg/kg/day; both values assigned by reviewer
Maternal effects	Maternal survival was 100% in all groups. Slight increase in matted haircoat (primarily in nasal region) and slight reduction in fecal material beneath cages was noted in 50mg/kg/day rabbits. Occasional instances of nasal discharge, soft stool, hair loss and scabbing were noted in all groups during gestation. One 50mg/kg/day rabbit aborted on day 19 of gestation. Maternal body wt in treated rabbits at all doses were comparable to controls throughout treatment (gest. day 6-28) and gestation (day 0-29) periods. Mean maternal adjusted body wt (minus gravid uterus) at termination in all groups was comparable to controls. Pregnancy ratio was 87.5, 81.3, 81.3, 93.8 in 0, 10, 25 and 50mg/kg/day groups, respectively. Two control dams and one 50mg/kg/day dam had all resorptions. There were no biologically or statistically significant differences in mean number of corpora lutea, total implantations, early or late resorptions, postimplantation loss, viable fetuses, fetal sex index, or mean fetal body wt in any RTO treated group compared to controls.
Embryo/fetal effects	Average litter size was 6.1, 6.5, 6.4, and 5.9 and average fetal body wt (both sexes) was 38.9, 43.0, 42.5, and 42.4g in 0, 10, 25, and 50mg/kg/day groups, respectively. There were no biologically or statistically significant differences in number of litters with malformations (external, soft tissue, skeletal) in any treated group compared to controls: 5/12 litters (85 pups), 1/13 litters (84 pups), 3/13 litters (83 pups) and 5/13 litters (82 pups examined) in 0, 10, 25, and 50mg/kg/day, respectively. In the 50mg/kg/day group, one occurrence of atlas-occipital anomaly and one occurrence of enlarged heart with great vessel anomaly, were observed in 2 separate litters. Scoliosis was present in all groups including control, with slightly higher incidence in the 50mg/kg/day group., but incidences were within the range of historical control data for this laboratory. Fetuses and litters with genetic or developmental variations were comparable in all groups.
Conclusions (study authors)	Rerun tower overhead did not produce a teratogenic response in pregnant New Zealand White rabbits when administered orally in corn oil vehicle at dose levels of 10, 25 and 50mg/kg/day. With the exception of one 50mg/kg/day female that aborted, minimal maternal toxicity was observed at any dose level.
Data Quality *Reliabilities*	**2.** Reliable with restrictions. Analysis of test article concentration in corn oil vehicle was not performed.
References	Schardein, J.L. 1981. Teratology study in rabbits: Rerun tower overheads (MRTC-171-79) IRDC #450-011. International Research and Development Corp., Mattawan, MI. for Mobil Petrochemicals Division, Edison, NJ
Other	
Last *changed*	2/28/2002 (Prepared by a contractor to the Olefins Panel)